

Express Mail No.: EV889156198US
International Application No.: PCT/IN2003/000440
International Filing Date: 31 December 2003
Preliminary Amendment

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-24. (Canceled)

25. (New) An antioxidant composition comprising a dichloromethane extract having antioxidant activity that is obtained from medulla and peel of tuberous roots of *Decalepis hamiltonii*.

26. (New) A pharmaceutical composition comprising the antioxidant composition of claim 25 and at least one pharmaceutically acceptable excipient.

27. (New) A method for preparing an antioxidant composition, comprising:

extracting medulla and peel of a *Decalepsis hamiltonii* tuberous root with dichloromethane to obtain an extract having antioxidant activity, and thereby preparing the antioxidant composition.

28. (New) The method of claim 27 which further comprises concentrating the extract.

29. (New) The method of claim 27 wherein the *Decalepsis hamiltonii* tuberous root is surface sterilized by washing with 70% alcohol.

30. (New) The method of claim 27 wherein in the step of extracting dichloromethane is present at a ratio of about 2:1 to the tuberous root by weight.

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31. (New) The method of claim 27 wherein the antioxidant activity ranges from 4-47% in an assay of antioxidant activity that comprises evaluating β -carotene bleaching, wherein % antioxidant activity is determined according to the formula:

$$\% \text{ antioxidant activity} = 100[1 - (A^0 - A') / (A^0 - A'0)]$$

wherein:

A^0 = zero time absorbance at 470 nm of a sample comprising the extract having antioxidant activity and an oxygenated aqueous β -carotene linoleic acid emulsion,

A' = absorbance at 470 nm of the sample after incubation for a time period t ,

$A'0$ = zero time absorbance at 470 nm of a control comprising the oxygenated aqueous β -carotene linoleic acid emulsion without the extract, and

$A'0$ = absorbance at 470 nm of the control after incubation for the time period t .

32. (New) A method for preparing an antioxidant composition, comprising:

extracting medulla and peel of a *Decalepsis hamiltonii* tuberous root with dichloromethane to obtain an extract having antioxidant activity, wherein the antioxidant activity comprises free radical scavenging activity; and

concentrating the extract, and thereby preparing the antioxidant composition.

33. (New) The method of claim 32 wherein the free radical scavenging activity comprises hydroxyl radical scavenging activity.

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34. (New) The method of claim 32 wherein the antioxidant composition is selected from:

(a) an antioxidant composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm, the composition having antioxidant activity of 30 to 45% when evaluated in an assay that comprises evaluating β -carotene bleaching, wherein % antioxidant activity is determined according to the formula:

$$\% \text{ antioxidant activity} = 100[1 - (A^0 - A') / (A^0 - A'0)]$$

wherein:

A^0 = zero time absorbance at 470 nm of a sample comprising the extract having antioxidant activity and an oxygenated aqueous β -carotene linoleic acid emulsion,

A' = absorbance at 470 nm of the sample after incubation for a time period t ,

$A'0$ = zero time absorbance at 470 nm of a control comprising the oxygenated aqueous β -carotene linoleic acid emulsion without the extract, and

$A''0$ = absorbance at 470 nm of the control after incubation for the time period t ,

(b) an antioxidant composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm, the composition having antioxidant activity of 35 to 46% when tested in an assay of antioxidant activity that comprises determining free radical scavenging activity by measuring absorbance at 517 nm of a methanolic solution of α,α -diphenyl- β -picryl hydrazyl, wherein % free radical scavenging activity is determined according to the formula:

$$\% \text{ free radical scavenging activity} = [(A_c - A_s) / A_c] \times 100,$$

wherein:

A_c is absorbance at 517 nm of a methanolic α,α -diphenyl- β -picryl hydrazyl solution without the extract, and

A_s is absorbance at 517 nm of a methanolic α,α -diphenyl- β -picryl hydrazyl solution with the extract,

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(c) an antioxidant composition in which the extract is present in a concentration range of 100 ppm to 200 ppm, the composition having antioxidant activity of 36 to 47% when tested in an assay of antioxidant activity that comprises determining hydroxyl scavenging activity by detecting a percentage of formaldehyde that is formed from hydroxyl radical-induced oxidation of DMSO when the extract is present relative to formaldehyde that is formed from hydroxyl radical-induced oxidation of DMSO when the extract is absent,

(d) an antioxidant composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm and that has antioxidant activity of 36 to 47% in the assay of antioxidant activity as in (a),

(e) an antioxidant composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm and that has antioxidant activity of 32 to 48% in the assay of antioxidant activity as in (b), and

(f) an antioxidant composition in which the extract is present in a concentration range of 100 ppm to 200 ppm and that has antioxidant activity of 43 to 49% in the assay of antioxidant activity as in (c).

35. (New) A method for preparing an antioxidant pharmaceutical composition, comprising:

extracting medulla and peel of *Decalepsis hamiltonii* tuberous roots with dichloromethane to obtain an extract having antioxidant activity; and

mixing the extract having antioxidant activity with a pharmaceutically acceptable excipient or an edible item, and thereby preparing the antioxidant pharmaceutical composition.

36. (New) The method of claim 35 wherein the antioxidant activity comprises free radical scavenging activity.

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37. (New) The method of claim 36 wherein the free radical scavenging activity comprises hydroxyl radical scavenging activity.

38. (New) The method of claim 35 wherein the antioxidant activity ranges from 4-47% in an assay of antioxidant activity that comprises evaluating β -carotene bleaching, wherein % antioxidant activity is determined according to the formula:

$$\% \text{ antioxidant activity} = 100[1 - (A^0 - A') / (A^0 - A'0)]$$

wherein:

A^0 = zero time absorbance at 470 nm of a sample comprising the extract having antioxidant activity and an oxygenated aqueous β -carotene linoleic acid emulsion,

A' = absorbance at 470 nm of the sample after incubation for a time period t ,

$A'0$ = zero time absorbance at 470 nm of a control comprising the oxygenated aqueous β -carotene linoleic acid emulsion without the extract, and

$A''0$ = absorbance at 470 nm of the control after incubation for the time period t .

39. (New) The method of claim 35 wherein the antioxidant pharmaceutical composition is selected from the group consisting of

(a) a pharmaceutical composition in which the extract is present in a concentration range of 100 ppm to 1,000 ppm,

(b) a pharmaceutical composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm, the pharmaceutical composition having antioxidant activity of 30 to 45% when evaluated in an assay that comprises evaluating β -carotene bleaching, wherein % antioxidant activity is determined according to the formula:

$$\% \text{ antioxidant activity} = 100[1 - (A^0 - A') / (A^0 - A'0)]$$

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wherein:

A^0 = zero time absorbance at 470 nm of a sample comprising the extract having antioxidant activity and an oxygenated aqueous β -carotene linoleic acid emulsion,

A' = absorbance at 470 nm of the sample after incubation for a time period t ,

A'^0 = zero time absorbance at 470 nm of a control comprising the oxygenated aqueous β -carotene linoleic acid emulsion without the extract, and

A'^0 = absorbance at 470 nm of the control after incubation for the time period t ,

(c) a pharmaceutical composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm, the pharmaceutical composition having antioxidant activity of 35 to 46% when tested in an assay of antioxidant activity that comprises determining free radical scavenging activity by measuring absorbance at 517 nm of a methanolic solution of α,α -diphenyl- β -picryl hydrazyl, wherein % free radical scavenging activity is determined according to the formula:

$$\% \text{ free radical scavenging activity} = [(A_c - A_s)/ A_c] \times 100,$$

wherein:

A_c is absorbance at 517 nm of a methanolic α,α -diphenyl- β -picryl hydrazyl solution without the extract, and

A_s is absorbance at 517 nm of a methanolic α,α -diphenyl- β -picryl hydrazyl solution with the extract,

(d) a pharmaceutical composition in which the extract is present in a concentration range of 100 ppm to 200 ppm, the pharmaceutical composition having antioxidant activity of 36 to 47% when tested in an assay of antioxidant activity that comprises determining hydroxyl scavenging activity by detecting a percentage of formaldehyde that is formed from hydroxyl radical-induced oxidation of DMSO when the extract is present relative to formaldehyde that is formed from hydroxyl radical-induced oxidation of DMSO when the extract is absent,

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(e) a pharmaceutical composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm and that has antioxidant activity of 36 to 47% in the assay of antioxidant activity as in (b),

(f) a pharmaceutical composition in which the extract is present in a concentration range of 500 ppm to 1,000 ppm and that has antioxidant activity of 32 to 48% in the assay of antioxidant activity as in (c), and

(g) a pharmaceutical composition in which the extract is present in a concentration range of 100 ppm to 200 ppm and that has antioxidant activity of 43 to 49% in the assay of antioxidant activity as in (d).